UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Note to Reader
January 8, 1998

Background: As part of its effort to involve the public in the implementation of the Food Quality Protection Act of 1996 (FQPA), which is designed to ensure that the United States continues to have the safest and most abundant food supply. EPA is undertaking an effort to open public dockets on the organophosphate pesticides. These dockets will make available to all interested parties documents that were developed as part of the U.S. Environmental Protection Agency's process for making reregistration eligibility decisions and tolerance reassessments consistent with FQPA. The dockets include preliminary health assessments and, where available, ecological risk assessments conducted by EPA, rebuttals or corrections to the risk assessments submitted by chemical registrants, and the Agency's response to the registrants' submissions.

The analyses contained in this docket are preliminary in nature and represent the information available to EPA at the time they were prepared. Additional information may have been submitted to EPA which has not yet been incorporated into these analyses, and registrants or others may be developing relevant information. It's common and appropriate that new information and analyses will be used to revise and refine the evaluations contained in these dockets to make them more comprehensive and realistic. The Agency cautions against premature conclusions based on these preliminary assessments and against any use of information contained in these documents out of their full context. Throughout this process, If unacceptable risks are identified, EPA will act to reduce or eliminate the risks.

There is a 60 day comment period in which the public and all interested parties are invited to submit comments on the information in this docket. Comments should directly relate to this organophosphate and to the information and issues available in the information docket. Once the comment period closes, EPA will review all comments and revise the risk assessments, as necessary.

These preliminary risk assessments represent an early stage in the process by which EPA is evaluating the regulatory requirements applicable to existing pesticides. Through this opportunity for notice and comment, the Agency hopes to advance the openness and scientific soundness underpinning its decisions. This process is designed to assure that America continues to enjoy the safest and most abundant food supply. Through implementation of EPA's tolerance reassessment program under the Food Quality Protection Act, the food supply will become even safer. Leading health experts recommend that all people eat a wide variety of foods, including at least five servings of fruits and vegetables a day.

Note: This sheet is provided to help the reader understand how refined and developed the pesticide file is as of the date prepared, what if any changes have occurred recently, and what new information, if any, is expected to be included in the analysis before decisions are made. **It is not meant to be a summary of all current information regarding the chemical.** Rather, the sheet provides some context to better understand the substantive material in the docket (RED chapters, registrant rebuttals, Agency responses to rebuttals, etc.) for this pesticide.

Further, in some cases, differences may be noted between the RED chapters and the Agency's comprehensive reports on the hazard identification information and safety factors for all organophosphates. In these cases, information in the comprehensive reports is the most current and will, barring the submission of more data that the Agency finds useful, be used in the risk assessments.

Jack E. Housenger, Acting Director
Special Review and Reregistration Division

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

10/28/98

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: Health Effects Division Toxicity Chapter for Disulfoton for Reregistration Eligibility Decision (RED).

DP Barcode: D250600
Rereg Case: 0102

PC Code: 032501
Cas Reg No.: 274-04-4
Caswell File No.: 341

From: David G Anderson, Toxicologist
Reregistration Branch-2
HED (7509C)

To: Betty Shackleford/Phip Poli PM 53
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SRRD (7507C)

Thru: Alan Nielsen, Branch Senior Scientist
Reregistration Branch-2
HED (7509C)

The following is the Toxicity Chapter for the RED for disulfoton.

EXECUTIVE SUMMARY:

Disulfoton is classified as acutely toxic, toxicity category I, by the oral, dermal and inhalation routes. Disulfoton was too toxic for guideline studies on primary eye, skin irritation and dermal sensitization to be conducted, thus the data requirements were waived.

The mode of action of disulfoton is inhibition of cholinesterase. In all of the studies evaluated in this hazard assessment, the LOEL and NOEL were established through the inhibition of cholinesterase (the basis for all regulatory endpoints). Clinical signs, such as muscle fasciculation and tremors are seen either at higher dose levels or at the LOEL for some studies. All three cholinesterases (plasma, erythrocyte and brain) are inhibited at the lowest dose tested and are likely to occur across species. There are slight species differences, but the differences may be due to normal variation and differences in the duration of the studies conducted in different species. Adult females appear to be slightly more sensitive, and in a 6-month study in rats (MRID# 43058401), cholinesterase inhibition was seen only in females.

The cholinesterase endpoints between acute and chronic studies in rats all are within a 10 fold exposure level. Longer exposure always showing cholinesterase inhibition at lower dose levels. Clinical signs occurred at the same dose level as cholinesterase inhibition in the acute neurotoxicity study, whereas in the 90-day neurotoxicity study, cholinesterase inhibition occurred at a lower dose level. Motor activity was affected at lower dose levels in the 90-day study than in the acute study, but no treatment related or significant neuropathology occurred either acutely or in the 90-day studies.

There is no increased susceptibility to fetuses or pups in acceptable developmental and reproductive toxicity studies in the rabbit or rat. Pup death occurred at the highest dose tested. The deaths were attributed to an inadequate milk supply and maternal care failure. In the developmental toxicity study in the rat, developmental toxicity occurred at higher doses than caused toxicity in dams. Developmental toxicity in the rat was seen in the form of incomplete ossification, but no developmental toxicity was seen in the rabbit at the dose levels administered. In the study on reproduction, cholinesterase was inhibited (plasma, erythrocyte and brain) in parents at lower dose levels than in pups.

No obvious endocrine disruption was seen in any of the studies. Absolute testes and ovarian weights were decreased (of unknown cause) at the highest dose levels and in the presence of cholinesterase inhibition in the chronic rat study, which may be endocrine mediated. However, these could not be unequivocally attributed to endocrine effects.

There is an adequate dermal absorption study in rats and an adequate 21-day dermal study in rabbits showing cholinesterase inhibition (plasma, erythrocyte and brain).

There are no carcinogenicity concerns in two acceptable studies in the rat and mouse. An adequate dose level was reached in the study in rats to test the carcinogenic potential of disulfoton, based on decreased body weights and body weight gains. In mice, the highest dose tested in this study is approximates 35% of the LD_{50} and higher dietary concentrations would have resulted in significant compound-related mortality of the test animals. Thus, the dose levels were considered adequate to test the carcinogen potential of disulfoton in mice.

Disulfoton is positive in some mutagenicity studies without activation, but negative or weakly positive in most with activation. With no carcinogenicity concerns and no reproductive toxicity concerns at relevant dose levels, the mutagenicity concerns are low. The mutagenicity data base is complete for the pre-1990 required three mutagenicity categories and the *in vivo* data base support a lack of concern for the mutagenicity of disulfoton.

The metabolism of disulfoton was studied in the rat. It was found to be rapidly absorbed and excreted with over 95% of the administered C^{14} labeled disulfoton being recovered in the urine and approximately 90% excretion within 24 hours. Less than 2% was recovered from the feces. Bioaccummulation was not observed with less than 0.3% being recovered in tissues and less than 1% being recovered in the carcass. A major metabolite was incompletely identified, but it co-chromatographed with 1-(ethylsulfonyl)-2-(methylsulfonyl)ethane, a fully oxidized form of the putative hydrolysis product. The toxic metabolites of disulfoton are disulfoton sulfoxide, disulfoton sulfone, disulfoton oxygen analog (demeton-S), disulfoton oxygen analog sulfoxide and disulfoton oxygen analog sulfone. The Metabolism Committee determined that the raw agriculture commodity,

meat, diary and poultry product residues to be regulated are disulfoton, disulfoton oxygenated analog and their sulfoxides and sulfones.

THE TOXICTY DATA BASE FOR DISULFOTON:

Acute Toxicity (81-1 to 8)

Disulfoton is acutely toxic (Toxicity category I) with an oral LD50 of 1.9 mg/kg for female rats. The dermal LD50 is 3.6 mg/kg for female rats. Note at the LD50, apparently greater than 50% of dermaly applied disulfoton is absorbed, while at lower concentrations only 36% is absorbed. The data requirements for primary eye irritation, dermal irritation and dermal sensitization were waived because of the acute toxicity of disulfoton.

Acute Toxicity of <u>disulfoton</u>, technical

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral	Acc# 072293,Doc# 003958,p41	LD_{50} = M: 6.2 mg/kg; F:1.9 mg/kg	I
81-2	Acute Dermal	Acc# 07793, Doc# 03958,p71 & 004223,p24	LD_{50} = M: 15.9 mg/kg; F: 3.6 mg/kg	I
81-3	Acute Inhalation	Acc# 258569, Doc# 05789	LC_{50} = M: 0.06 mg/L; F: 0.015 mg/L	I
81-4	Primary Eye Irritation	Data requirement waived. Doc# 03958,p12; 004223,p14		
81-5	Primary Skin Irritation	Data requirement waived. Doc# 03958,p12;004223.p14		
81-6	Dermal Sensitization	Data requirement waived. Doc# 03958,p12		
81-7	Acute Delayed Neurotoxicity	MRID# 00129384 Doc# 012484	Equivocal, study to be repeated	
81-8	Acute Neurotoxicity	42755801	Reversible neurotoxic signs consistent with the cholinesterase inhibition 1.5 mg/kg in females and 5.0 mg/kg in males	

Acute Inhalation Study/Rats (81-3)

CITATION: Anonomus (1978) Acute and 5-Day Inhalation in the rat with disulfoton. Study laboratory: Bayer AG Instit. Study# 7827. Date: 9/27/78.
Accession# 258569. Unpublished.

Executive Summary: Disulfoton, technical (94.4%) was administered to 20 Wistar rats/sex/group at 0, 34, 48, 51, 64, 78 or 96 μg/L for males and 0, 3.4, 5, 7, 10, 13 or 20 μg/L for females for 4 hours in a nose only experiment (MRID No.: Accession# 258569). The NOEL for death was 34 μg/L for males and 3.4 μg/L for females. LC50 for males was 60 μg/L with animals dying at $\geq$ 48 μg/L. The LC50 for females was 15 μg/L with animals dying at $\geq$5 μg/L.

In addition, 10 rats/sex were administered disulfoton for 4 hour/day for 5 days by inhalation at 0, 0.5, 1.8 or 9.8 μg/L in a nose only exposure; the following cholinesterase inhibition studies were conducted on 5 rats/sex/group after one of the five 4 hour exposures in the 5 day study. After 1 exposure in males, plasma cholinesterase inhibition ($\geq$17%) occurred at $\geq$1.8 μg/L and erythrocyte cholinesterase inhibition ($\geq$15%) occurred at 9.8 μg/L. After 1 exposure in females, plasma cholinesterase inhibition ($\geq$40%) occurred at $\geq$1.8 μg/L and erythrocyte cholinesterase inhibition ($\geq$23%) occurred at $\geq$9.8 μg/L.

After 3 to 5 exposures in males, plasma cholinesterase inhibition was reduced ($\geq$40%) and erythrocyte cholinesterase inhibition ($\geq$16%) at $\geq$1.8μg/L. After 3 to 5 exposures in females, plasma cholinesterase inhibition was reduced ($\geq$31%) at $\geq$0.5 μg/L and erythrocyte cholinesterase inhibition was reduced ($\geq$17%) at $\geq$1.8 μg/L. No deaths occurred after one 4 hours exposure at 9.8 μg/L in either males or females, however, deaths occurred in females after the 3rd exposure at 9.8 μg/L.

The acute inhalation NOEL/LOEL for males and females are 0.0005/0.0018 mg/L based on increased plasma cholinesterase inhibition and NOEL/LOEL of 0.0018/0.0098 mg/L for males and females based on increased erythrocyte cholinesterase inhibition after 1 exposure.

After 3 to 5 exposures, males showed NOEL/LOEL of 0.0005/0.0018 mg/L based on increased plasma and erythrocyte cholinesterase inhibition. Females showed NOEL/LOEL of <0.0005/0.0005 mg/L based on increased plasma cholinesterase inhibition after 3 to 5 exposures and the NOEL/LOEL are 0.0005/0.0018 mg/L based on increased erythrocyte cholinesterase after 3 to 5 exposures.

The study is acceptable under Guideline 81-3 for acute inhalation in rats.

Acute Delayed Neurotoxicity in Hens (81-7):

CITATION: Hixson, EJ (1983) Acute Delayed Neurotoxicity Study on Disulfoton. Laboratory: Mobay Chemical Corp., Metcalf, Stilwell, KS. Study number 82-418-01 (Mobay# 82655). March 7, 1983). (MRID# 00129384). Unpublished.

EXECUTIVE SUMMARY: Disulfoton (97.8% pure) was administered by gavage at 30 mg/kg to 20 hens; 0.5 mg/kg of atropine was administered (im) 10 minutes before the disulfoton dose and 12.5 mg/kg of PAM-2 was administered (im) 30 minutes after the disulfoton dose (MRID# 00129384). This dosing regimen was repeated at day 22. Five hens were used as a negative control. Five hens were administered atropine and PAM-2 (but no disulfoton) similarly to the disulfoton dosed group as an atropine and PAM-2 control and 10 hens were dosed with tri-O-cresol phosphate (500 mg/kg) as a positive control group. The 30 mg/kg dose level was shown to be lethal to hens without atropine administration. Samples of sciatic nerve, spinal cord (cervical, thoracic and lumbar) and brain (mid-brain, brain stem and cerebellum) were fixed in formalin and histological examination conducted.

The tri-O-cresol phosphate positive control group exhibited typical delayed neurotoxicity.

Pharmacologic signs were observed (loss of equilibrium, decreased activity, diarrhea and locomotor ataxia) in 14/20 hens after the first treatment, which subsided by day 5, except in one hen demonstrating ataxia and torticollis which decreased by day 15. These signs were considered by the report authors to be due to acute effects of disulfoton and not due to delayed neurotoxicity.

Body weight of the disulfoton group (91% of the negative control and 94% of the atropine and PAM-2 treated control) and atropine and PAM-2 groups (97% of control) were lower than control hens at termination.

Neuropathy in the form of degeneration digestion chambers (18/20 disulfoton treated hens versus 9/10 combined control hens), all grade 1 except one grade 2 pathology was seen at the thoracic level in a control hen, neuronal degeneration all grade 1 in (5/20 disulfoton hens versus 1/10 combined control hens, all grade 1) and axonal swelling all grade 1 (6/20 disulfoton hens versus 5/10 combined control hens) and demyelination all grade 1 (0/20 disulfoton treated hens versus 1/10 combined control hens). Macrophage accumulation occurred in 17/20 (85%) disulfoton treated hens versus 7/10 (70%) combined control hens. Macrophage accumulation an/or lymphocyte accumulation occurred in 4/5 of the disulfoton treated hens and in 1/10 of the combined control hens with neuronal degeneration. However, this accumulation was not always noted at the same site as the neuronal degeneration. This inflammation in old hens adds uncertainty to the effects seen in the study. **The study is suggestive but equivocal for delayed neurotoxic effects.**

The study is down graded from acceptable to unacceptable and not upgradable for an acute delayed neurotoxicity study in hens (81-7). Due to the equivocal but suggestive nature of the neurotoxic effects and the use of older hens. The study should be repeated using 8-14 month old hens. SignOff Date: 2/12/1998; DP Barcode: D241669; HED DOC Number: 012484.

The HAZARD ID SARC for disulfoton recommended that a DCI be issued for an Acute Delayed Neurotoxicity in hens (81-7) with an added NTE study with disulfoton. These new studies are requested because 17 month old hens instead of 8 to 14 month old hens were used and neuronal degeneration (5/20 versus 1/10 in controls, all grade 1) was seen, which were considered suggestive but equivocal (because of the age of the hens) for neuronal effects. Depending on the results from these new studies, additional studies may be required.

Subsequent to a HAZARD ID SARC meeting on disulfoton, a neurotoxicology subgroup of the HAZARD ID SARC reviewed the original acute delayed neurotoxicity study in hens (MRID#

00129384) with disulfoton. They considered the study data was suggestive of organophosphate induced delayed neuropathy (OPIDN) and recommended that another study should be conducted. The FQPA Safety Factor Committee reduced the 10X UF to 3 due to the suggestive nature of OPIDN and until a fully acceptable and negative Acute Delayed Neurotoxicity study in hens with NTE study are submitted and evaluated. The DER (TOX# 004698) for the study, which had classified the study as acceptable, had raised questions about its acceptability in the HAZARD ID SARC meeting. A new Executive Summary has been prepared indicating that the hen study has been reclassified from acceptable to unacceptable not upgradable.

Acute Neurotoxicity/Rat (81-8)

Executive Summary: In an acute neurotoxicity screening study, disulfoton (97.8% pure) was administered in a single gavage dose to 10 male Sprague-Dawley rats at doses of 0, 0.25, 1.5, or 5.0 mg/kg and to 10 female Sprague-Dawley rats at doses of 0, 0.25, 0.75 or 1.5 mg/kg (MRID# 42755801). These rats were assessed for reactions in functional observational battery (FOB) and motor activity measurements at approximately 90 minutes post-dosing and on days 7 and 14. Cholinesterase determinations (erythrocyte and plasma) were made at 24 hours post-dosing. Six rats/sex/dose were examined for neuropathological lesions.

At 0.75 mg/kg, 4/10 females had muscle fasciculations. At 1.5 mg/kg, males had muscle fasciculations, diarrhea, and sluggishness and females also had tremors, ataxia, oral staining, decreased activity/sluggishness, decreases in motor and locomotor activity (38–49% of control), and a slightly increased duration of nasal staining. One female at 1.5 mg/kg died from cholinergic intoxication on the day of dosing. At 5.0 mg/kg, males also had symptoms similar to those observed in females at 1.5 mg/kg/day, including reduced motor/locomotor activity (36–45% of control). Recovery appeared to be complete in surviving animals by Day 14. **Based on the evidence of neurotoxicity (probably associated with inhibition of cholinesterase) in females at 0.75 mg/kg, the study LOEL is 0.75 mg/kg and the study NOEL is 0.25 mg/kg.**

At 0.75 mg/kg in females, cholinesterase activities were inhibited by 53% (erythrocyte) and 30% (plasma) and by 75% (erythrocyte) and 52% (plasma) at 1.5 mg/kg in females. At 5.0 mg/kg in males, cholinesterase activities were inhibited by 21% (erythrocyte) and 25% (plasma). **The LOEL for inhibition of cholinesterase activity is 0.75 mg/kg and the NOEL for inhibition of cholinesterase activity is 0.25 mg/kg.**

This study is classified as core-minimum and satisfies the guideline requirement for an acute neurotoxicity screen (81-8).

Subchronic Inhalation/Rats (82-4)

CITATION: Shiotsuka, RN (1989) Subchronic inhalation study of technical grade disulfoton (Di-Syston®) inhalation in rats. Testing Lab: Mobay Corp. Study# 88-141-AU/99648. Date: 7/31/89. MRID# 41224301. Unpublished study.

Executive Summary: Disulfoton was administered by inhalation to 12 Fisher 344 rats per sex per group for air control, polyethylene glycol-400: 50% ethanol vehicle control, 0.015, 0.15 or 1.5 mg/m^3 nominal dose levels for 90-days in a nose only chamber (MRID No.: 41224301). The analytical determined mean dose levels were 0, 0, 0.018, 0.16 and 1.4 mg/m^3 for male and female rats. The rats were exposed to the test material 6 hours per day, 5 days per week. The particle sizes in the inhalation chambers had a MMAD ± geometric standard deviation of 1.3 ± 1.4, 1.1 ± 1.3, 1.0 ± 1.3 and 1.1 ± 1.4 μm for the two controls, 0.015, 0.15 and 1.5 mg/m^3 nominal dose levels, respectively. The range in mean daily particle sizes had a MMAD of 0.5 ± 1.0 μm to 2.6 ± 1.6 μm.

At the highest dose level, plasma cholinesterase was depressed in males (19% and 14% from air controls at 38 days and term, respectively, $p \leq 0.05$) and in females (27% and 31% from air controls at 38 days and term, respectively, $p \leq 0.05$). Brain cholinesterase was depressed in males (29%) and females (28%) at termination. Erythrocyte cholinesterase was depressed in females at 38 days (11% at 38 days, $p \leq 0.05$, not considered biologically relevant) at 0.16 mg/m^3 and higher in males and females at 1.4 mg/m^3 at 38 days and term. Brain cholinesterase were depressed (10%, $p \leq 0.05$) at 0.16 mg/m^3, but this degree of variation was not considered biologically relevant due to variation noted in this parameter. Inflammation of the male nasal turbinates occurred at 1.4 mg/m^3. No other test material related effects were noted. **The NOEL/LEL is 0.16 mg/m^3/1.4 mg/m^3 or 0.00016/0.0014 mg/L for plasma, erythrocyte and brain cholinesterase depression.**

Core classification: Guideline. The study (MRID# 41224301) is acceptable under guideline 82-4 for a 90-day inhalation study in rats.

Comments about study and/or endpoint: This study also has cholinesterase inhibition data for day 37.

21-Day Dermal Toxicity/Rabbits (82-5)

CITATION: Flucke, W. (1986) Study of Subacute Dermal Toxicity to Rabbits. Bayer AG, Fachbereich Toxikologie, Wuppertal - Elberfeld, F.R. Germany. Study No.:14747. June 20, 1986. MRID 00162338. Unpublished.

EXECUTIVE SUMMARY: In a repeated dose dermal toxicity study (MRID 00162338) S276 Technical disulfoton (97.8% a.i., Batch No. 79-R-225-40), was applied to the shaved skin of 5 New Zealand White rabbits/sex/dose at dose levels of 0, 0.4, 1.6 or 6.5 mg/kg, 6 hours a day, 5 days/week for 15 days. Doses were selected based on a preliminary range-finding study in which clinical signs of cholinergic intoxication and death at 10 mg/kg/day following 1 or 2 applications. Slight inhibition of plasma ChE inhibition at 2 mg/kg and no effect on plasma or RBC ChE inhibition at 0.4 mg/kg. Plasma and RBC ChE were determined at study initiation, day 6, 11, and termination. Brain ChE was determined at termination.

Repeated dermal application of disulfoton or vehicle (Cremophor EL in sterile saline) 6 hours a day for 15 days had no effect on hematology, clinical chemistry, urinalysis, gross pathology and

absolute and relative organ weights. There was no dermal reaction to repeated dermal application. **Systemic Toxicity** was observed in high-dose males and females as a marked reduction in food consumption and body weights and death ensuing within 1 to 2 weeks of initiation of treatment. The **Systemic Toxicity NOEL = 1.6 mg/kg/day** and **LOEL = 6.5 mg/kg/day**, based on reduced food consumption and weight gain.

At the highest dose, all males and females died or were sacrificed following ≈ 6 days of treatment due to acute cholinergic signs such as muscle spasms, dyspnea and salivation. In one high dose male which survived 6 treatments, plasma (75%) and RBC (31%) Cholinesterase was depressed. Plasma ChE of mid-dose males (17 - 24%) and females (31 - 44%) depressed; RBC ChE of males (15 - 19%) and females (7 - 33%) was depressed, compared to concurrent controls. Brain ChE of males and females was depressed 7 - 8%. The ChE **NOEL = 0.4 mg/kg/day and LOEL = 1.6 mg/kg/day**, based on inhibition of plasma and RBC ChE and marginal inhibition of brain ChE.

The study is classified as **Acceptable** and satisfies the guideline requirement for a subchronic dermal toxicity study (82-2) in rabbits.

Subchronic Neurotoxicity Study/Rats (82-7)

CITATION: L.P. Sheets and B.F. Hamilton (1993) A subchronic dietary neurotoxicity screening study with technical grade disulfoton (Di-Syston®) in Fischer 344 rats. Testing lab.: Miles Inc. Study# 92-472-NS (106332). Date: 9/23/1993. MRID# 42977401. Unpublished study.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID# 42977401), disulfoton (98.7–99.0% pure) was administered in the diet to 12 male and 12 female Fischer 344 rats at dietary levels of 0, 1, 4, or 16 ppm (0, 0.063, 0.270, and 1.08 mg/kg/day in males and 0, 0.071, 0.315, and 1.31 mg/kg/day in females). Of these 12 rats/sex/dose, 6/sex/dose were used for a neurohistopathological examination at the end of the study.

At 4 ppm, females had muscle fasciculations, urine staining, and increased food consumption (approximately 110% of control). At 16 ppm, both males and females had increased reactivity, perianal staining, tremors, increased defecation, decreased forelimb grip strength (37–86% of control), decreased motor and locomotor activity (39–71% of control), decreased body weight gain (81–83% of control), and corneal opacities. At 16 ppm, males also had muscle fasciculations and appeared sluggish, and one female died due to cholinergic intoxication. **The study LOEL is 4 ppm (0.315 mg/kg/day) and the study NOEL is 1 ppm (0.071 mg/kg/day), based on clinical signs in females consistent with neurotoxicologic effects of cholinesterase inhibition.**

Erythrocyte, plasma, and brain cholinesterase activities were inhibited by 15–23%, 59–80%, and 87–100% in females at 1, 4, and 16 ppm, respectively, and 20–67% and 66–100% in males at 4 and 16 ppm, respectively. Males at 1 ppm had a statistically significant inhibition of erythrocyte cholinesterase at 13 weeks (15% inhibition); other cholinesterase activities in males at 1 ppm were not significantly affected. **The LOEL for inhibition of cholinesterase activity is 1 ppm and the**

NOEL for inhibition of cholinesterase activity is less than 1 ppm.

This study is classified as core-guideline and satisfies the guideline requirement for a subchronic neurotoxicity screen (82-7).

Chronic Toxicity Studies/Dogs (83-1b)

CITATION: Jones, R.D. and T.F. Hastings (1997) Technical grade Disulfoton: A chronic toxicity feeding study in the Beagle dog. Bayer Corporation, Stillwell, KS. Study Number 94-276-XZ. Report No. 107499. February 5, 1997. MRID 44248002. Unpublished.

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 44248002), disulfoton (97% a.i.%) was administered orally in the diet to purebred beagle dogs (4/sex/dose) at dose levels of 0.5, 4 or 12 ppm (equivalent to 0.015, 0.121 and 0.321 mg/kg/day for males; and 0.013, 0.094 and 0.283 mg/kg/day for females) for one year. Potential ocular and neurologic effects were addressed.

Plasma cholinesterase was decreased starting at day 7 in the 4.0 ppm dose groups of the study through to termination (males 39% to 46%; females 32% to 45%). Erythrocyte cholinesterase was decreased starting at day 91 in the 4.0 ppm dose groups through to termination (males 23% to 48%; females 17% to 49%). Not all the values at 4.0 ppm were statistically significant, probably because of the wide range in values, but at least 2 animals per group showed biologically significant cholinesterase inhibition.

By termination cholinergic effects of the plasma, erythrocytes, brain, and ocular tissues were observed in both sexes in the 4 and 12 ppm treatment groups. Plasma and erythrocyte cholinesterase depression are compared to pretreatment values. Brain, cornea, retina and ciliary body cholinesterase depression are compared with concurrent control values at termination only. In the 12 ppm treatment groups, depressed cholinesterase was observed in plasma (56%-63%), erythrocytes (30%-91%), and brain (32%-33%) compared to their respective controls. In the 4 ppm treatment groups in males and females, cholinesterase was depressed in plasma (38%-46%), erythrocytes (40%- 38%), and brain (females only, 22%). Disulfoton inhibited cholinesterase of the cornea, retina, and ciliary body, but did not appear to alter the physiologic function of the visual system. In the 12 ppm treatment groups, depressed cholinesterase was observed in the cornea (60-67%), ciliary body (45-54%), and retina (males only; 67%). In the 4 ppm treatment groups, cholinesterase was inhibited in the cornea (50-60% lower), and retina (females only, 25%). No treatment-related ophthalmology findings or histological or electrophysiological changes in the retina were observed. No other treatment-related effects were observed. No animals died during the study. No treatment-related effects were observed in sytemic toxicity including food consumption, body weights, clinical signs, hematology, clinical blood chemistry or urinalysis parameters, electroretinograms, electrocardiogram or clinical neurological findings, organ weights or gross or microscopic post-mortem changes in any treatment group. No neoplastic tissue was observed in

dogs in the treatment and control groups. **The LOEL is 4 ppm (0.094 mg/kg/day), based on depressed plasma, erythrocyte, and corneal cholinesterase levels in both sexes, and depressed brain and retinal cholinesterase levels in females. The NOEL is 0.5 ppm (0.013 mg/kg/day). These LOEL/NOEL for plasma cholinesterase inhibition extend from day 7 to termination and for erythrocyte cholinesterase inhibition they extend from day 91 to termination.**

This study is classified **acceptable** and satisfies the Subdivision F guideline requirement for a chronic oral study in non-rodents (83-1b).

Chronic Toxicity Study/Dogs (83-1b)

CITATION: Hoffman, K.; Weischer, C.H.; Luchaus, G.; et al. (1975) S 276 (Disulfoton) Chronic Toxicity Study in Dogs (Two-year Feeding Experiment). Bayer, AG, W. Germany. Report No. 45287. December 15, 1976. MRID 00073348. Unpublished.

EXECUTIVE SUMMARY: In a chronic feeding study (MRID 00073348) Technical Di-Syston (95.7% a.i.) was administered in diet to 4 Beagles/sex/dose in the diet at dose levels of 0, 0.5, 1 or 2/5/8 ppm (0, 0.0125, 0.025 or 0.05/0.125/0.2 mg/kg/day, converted) for 104 weeks. In the high-dose group, 2 ppm was given for first 69 weeks, 5 ppm from 70 - 72 weeks, and 8 ppm from week 73 - termination. Body weights were determined weekly for 52 weeks, then biweekly until termination. Clinical evaluations to detect cholinergic signs, ophthalmological evaluations, hematology, clinical chemistries, urinalysis were performed on all animals pre-treatment, on weeks 13, 26, 39, 52, 65, 78, 91, and at termination. Plasma, and RBC cholinesterase was determined at 2-week intervals during the first 13 weeks and at about 3 month intervals thereafter. Brain cholinesterase was determined immediately after necropsy.

Treatment had no effects on general appearance and behavior, and toxic signs, ophthalmoscopy examinations, food consumption, body weight, hematology, clinical chemistry, organ weight and/or histopathology. At 2 ppm, plasma and RBC cholinesterase (ChE) was inhibited 50 and 33% in males and 22 and 36% in females, respectively, during week 40. Large fluctuations in plasma and RBC ChE inhibitions occurred until the dose was raised to 8 ppm. By the termination (104 weeks) of study, the plasma, RBC and brain ChE was inhibited 65, 58, and 34% in males and 49, 48 and 18% in females, respectively, compared to pre-treatment values. Based on the above, the **Systemic Toxicity NOEL = 2 ppm** (0.05 mg/kg/day) and **LOEL > 2 ppm**. The **cholinesterase NOEL = 1 ppm** (0.025 mg/kg/day) and **LOEL = 2 ppm** (0.05 mg/kg/day), based on plasma and RBC ChE inhibition.

The study is classified as **Acceptable** and **satisfies** the guideline requirement for a chronic toxicity study (83-1b) in the dog.

Carcinogenicity/Mice (83-2b)

CITATION: Hayes, R.H (1983) Oncogenicity study of disulfoton technical on mice. Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, KS. Study No. 80-271-04. August 10, 1983. MRID 00129456. Unpublished study.

EXECUTIVE SUMMARY: In a carcinogenicity toxicity study (MRID 00129456 & 00139598), disulfoton (98.2% a.i.) was administered to 50 Crl:CD-1 mice/sex/dose in the diet at dose levels of 0, 1, 4, or 16 ppm (0.15, 0.6, or 2.4 mg/kg/day, converted) for 108 weeks. In addition, 10 mice/sex/group were used as replacement animals. Cholinesterase activity in the plasma, RBC, and brain was determined at final sacrifice for 10 mice/sex randomly selected from the control and 16 ppm groups.

Treatment had no effect on bodyweights, food consumption, hematology, and mortality. Eight mice i.e., 1 male and 3 females from the 1 ppm group, 3 males from the 4 ppm group, and one male from the 16 ppm group, died during the first month and were replaced. Survival at 18 months ranged from 76 - 86% in all males, and 68 - 82% in all females. At termination survival ranged from 56 - 66% and 38 - 54%, in males and females, respectively. Cholinesterase (ChE) was markedly inhibited at the high-dose. In males, the plasma, RBC and brain ChE was inhibited 79, 56, and 44%; and in females it was inhibited 82, 50, and 46%, respectively, compared to controls. Enlarged spleen, liver, and lymph nodes were observed with greater frequency in females than males,; histologically diagnosed as lymphomas. The number of animals with malignant lymphoma, of all histologic cell types, were 10, 9, 12, and 15 in males and 27, 22, 26, and 34 in females, at 0, 1, 4, and 16 ppm, respectively. Tumor incidence lacked statistical significance by either the Chi-square or Fisher exact test. In high-dose females, absolute and relative kidney weights increased 22% and 11%, respectively, probably related to increased incidence of lymphomas in this organ. None of the increased organ weights/histopathological findings were considered treatment-related.

Based the above findings, the **Systemic Toxicity NOEL < 2.4 mg/kg/day and LOEL = 2.4 mg/kg/day**, based on plasma, RBC and brain ChE inhibition in males and females.

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate for testing the carcinogenic potential of disulfoton, even though, there was no clear indications of systemic toxicity such as body weight gains and liver specific enzymes. The highest dose tested in this study is approximates 35% of the LD_{50} and higher dietary concentrations would have resulted in significant compound-related mortality of the test animals.

The study is classified as **Acceptable**, and satisfies the guideline requirement for a oncogenicity study (83-2b) in mice.

Chronic feeding/Oncogenicity Study/Rats (83-5)

CITATION : Hayes, R.H (1985) Chronic feeding/oncogenicity study of technical disulfoton (Di-

SYSTON) with rats. Mobay Chemical Corporation, Stilwell, KS. Study No. 82-271-01. June 25, 1985. MRID #s 00146873. Unpublished.

and

Supplementary data upgrading MRID# 00146873 from supplementary to acceptable on the Harderian gland (MRID# 41850001) and optical and optic nerve lesions (MRID# 41850002).

EXECUTIVE SUMMARY: In a chronic feeding/carcinogenicity study (MRID # 00146873, 41850001, 41850002) Disulfoton (98.1% a.i., Batch No. 79-R-255-40) was administered to 60 Fischer 344 rats/sex/dose in the diet at dose levels of 0, 0.8, 3.3, or 13 ppm (0, 0.04, 0.165, or 0.650 mg/kg/day, converted by std. tables) for 105 weeks. Hematological determinations were done on 20/sex/dose and urine and blood chemistry on 10/sex/dose, randomly selected, at 0, 3, 6, 12, 18, and 24 months. Plasma and red cell cholinesterase (ChE) was determined on 10 rats/sex/dose at pre-treatment, 4, 14, 27, 53, 79 and 105 weeks and brain ChE at termination.

Administration of disulfoton in the diet up to 13 ppm had no effect on mortality, hematology, clinical chemistry and urine analysis. Mean body weights of high-dose rats were significantly depressed throughout the study. Body weight gains of high-dose males and females were depressed 29% and 48%, respectively, by termination when compared to the controls. At the mid and low dose, mean body weights of males were sporadically depressed, however, by the end of study the mean body weights were similar to controls. Females body weights were not effected at these dose levels. At 13 ppm, in females the absolute heart (9%) , liver (17%), and testes (24%) were decreased; in females the heart (13%), kidneys (13%), liver (27%) and ovaries (57%) decreased. Absolute brain weight was unchanged in males and females. In high-dose females the relative brain (59%), heart (33%), and kidneys (34%) increased, compared to the controls. Also, the relative lung (72%) and liver (9%) and brain (58%) weights were increased. At this dose the male relative brain weights were increased by 17%. None of the aforementioned organ weights were associated with any histopathology corroborative of toxicity. In high-dose males Harderian gland degenerative changes increased to 460% of controls and in females the elevation was dose-related (800, 1100 and 1633% of control values, respectively, all $p \leq 0.05$). Since there is no Harderian gland in the humans, the significance of pathological changes seen in the rat are uncertain. In addition, corneal vascularity (693% of control), corneal epithelial hyperplasia (1633% of control) and optic nerve degeneration (145% of control) were elevated in high-dose females and corneal vascularity (329% of control) in males. The eye histopathology was not affected in the mid and low doses. Based on the above, the **Systemic Toxicity NOEL = 0.8 ppm (0.04 mg/kg/day) and LOEL = 3.3 ppm (0.165 mg/kg/day)**, based on Harderian gland degeneration.

At termination, a dose-related inhibition in plasma, red cell and brain ChE was observed at all doses in both sexes. In males the plasma, red cell and brain ChE was inhibited 11%-94%, 19%-80%, and 16%-79%; and in females, it was 25%-95%, 12%-76%, and 21%-82%, respectively, compared to the controls. The **Cholinesterase NOEL < 0.8 ppm (0.04 mg/kg/day) and LOEL = 0.8 ppm (0.04 mg/kg/day)**, based on plasma, red cell and brain ChE inhibition in males and females. Starting at week 4 the LOEL in plasma ChE inhibition was 4 ppm (0.165 mg/kg/day) in males (27%) and females (64%) with a NOEL of 1 ppm (0.04 mg/kg/day). Starting at week 4 the LOEL in

erythrocyte ChE inhibition was increased at 1 ppm (0.04 mg/kg/day) (LDT) in males (16%) and females (30%) with no NOEL.

The maximum tolerated dose (MTD) was reached, based on decreased body weights and body weight gains and is considered adequate to test the carcinogenic potential of Disulfoton. Disulfoton treatment did not alter the spontaneous oncogenicity profile in both males and female Fischer 344 rats under the test conditions. In males and females, leukemia, adrenal cortex adenoma, adrenal pheochromocytoma, pituitary adenoma and carcinoma and thyroid-C cell adenoma was most frequently observed. Mammary gland fibroadenoma in both sexes, but most frequently in females. Testicular interstitial adenoma in males and stromal polyp of the uterus in females was observed. All these neoplasms were similar in type, time of onset, and incidence in both controls and disulfoton treated animals.

The study is classified as **Acceptable** and **satisfies** the guideline requirement for a chronic feeding/carcinogenicity study (83-5) in the rat.

Chronic Feeding/Oncogenicity Study/Rats (83-5)

CITATION: Carpy, S.; Klotzsche, C.; Cerioli, A. (1975) Disulfoton: 2-Year Feeding Study in Rats. Sandoz, Ltd., Switzerland. Report No. 47069. December 15, 1976. MRID 00069966. Unpublished.

EXECUTIVE SUMMARY: In a chronic feeding/carcinogenicity study (MRID 00069966) Technical Di-Syston® (95.7% a.i.) was administered to 60 SPF Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 0.5/5.0, 1.0 or 2.0 ppm (0, 0.0215/0.1900, 0.0456, or 0.0893 mg/kg/day in males and 0, 0.0267/0.1960, 0.0419 or 0.1033, mg/kg/day in females, respectively; calculated) for 104 weeks. The 0.5 ppm dose was fed for 81 weeks, then increased to 2 ppm because of no effects seen at the 1 ppm dose level. The rats in the 2 ppm group were initially maintained at 1.5 ppm for 4 - 5 weeks, then increased to full dose. Body weight, food consumption, food efficiency, hematology, clinical chemistries, and urinalysis were determined. Plasma, red cell and brain cholinesterase was determined from 5 overnight fasted animals/sex/group at termination. Necropsy was done on 10 animals/sex/dose; all others were examined for tumors. Histopathology was done on 5 animals/sex from the control and the 5 ppm group.

Treatment with Di-Syston did not effect, food consumption, body weight gain, hematology, clinical chemistry, and urinalysis. Mortality was high (20 - 37%) in females but lacked the dose response and no clear explanation was offered for cause of death; more than 1/3 of the dead animals autolyzed. At 0.5/5 ppm, in males the absolute/relative liver, spleen and kidney weights increased 12%/8%, 21%/17% and 23%/19%, respectively ($P \leq 0.05$); however, the histopathology of the organs were unremarkable. There was a trend for decreased absolute and relative brain weights in males and increased trend in females. The **Systemic Toxicity LOEL >1 ppm**.

Cholinesterase levels in plasma, red cells and brain was inhibited in males and females at two

higher doses and it was dose-related. At 2 ppm, the plasma, red cell and brain ChE of males was inhibited 14, 9.3, 9%, and in females 22, 13.3 and 17%, respectively, compared to the controls. At the 0.5/5 ppm dose, plasma, red cell and brain ChE of males and females was inhibited 20 - 39.6, 18.3 - 27.1 and 25 - 36%, respectively. ChE levels in the 1 ppm group males and females was not effects. The **ChE NOEL = 1 ppm and the LOEL = 2 ppm**, based on decreased plasma, red cell and brain cholinesterase levels.

The study is classified as **Unacceptable** and can not upgraded because multiple deficiencies in the conduct of the study and **does not satisfy** the guideline requirement for chronic toxicity/oncogenicity study (83-5) in the rat.

Developmental Toxicity Study in Rats (83-3)

CITATION: Lamb-DW and Hixson-EJ (1983) Embyrotoxic and teratogenic effects of Disulfoton. Study# 81-611-02 submitted by Mobay Chem. Corp. May 13, 1983. MRID#: 00129458. Unpublished Report.

EXECUTIVE SUMMARY: Disulfoton, technical (98.2%) was administered in a carbowax (polyethylene glycol 400) vehicle by gavage to 25 pregnant Sprague Dawley rats/group at 0, 0.1, 0.3 or 1.0 mg/kg/day from day 6 through day 15 of gestation (MRID# 00129458). On day 21, the rats were killed and 50% of each litter was examined for skeletal anomalies and the remainder for visceral anomalies. Cholinesterase inhibition studies on the dams at 21 days (2 weeks dosing) indicated an NOEL/LOEL of 0.1/0.3 mg/kg/day based on 41% inhibition of both plasma and erythrocyte cholinesterase. Fetuses showed incomplete ossification of the intraparietals and sternebrae at 1.0 mg/kg/day.

The NOEL/LOEL for maternal toxicity were 0.1/0.3 mg/kg/day based on 41% inhibition of both plasma and erythrocyte cholinesterase. The NOEL/LOEL for developmental toxicity were 0.3/1.0 mg/kg/day based on incomplete ossification of the intraparietals and sternebrae.

The study is acceptable under Guideline 83-3 for a developmental toxicity study in rats.

Developmental Toxicity in Rabbits (83-3)

CITATION: Tesh-JM et al. (1982) S276: Effects of oral administration upon pregnancy in the rabbit. An unpublished report (Bayer No. R 2351) prepared by Life Science Research, Essex, England and submitted to Bayer AG, Wuppertal, Germany. Dated December 22, 1982. MRID# 00147886. Unpublished Report.

EXECUTIVE SUMMARY: Disulfoton, technical was administered by gavage in a corn oil vehicle (5ml/kg) to 15, 14, 14 or 22 pregnant New Zealand White rabbits per group at 0, 0.3, 1.0 or 3.0 mg/kg/day, respectively from day 6 to 18 of gestation (MRID# 00147886). Since mortality and clinical signs were observed at 3.0 mg/kg/day, this dose level was reduced to 2.0 mg/kg/day and finally to 1.5 mg/kg/day. Analysis showed that the dosing solutions were 17, 14 and 10% below the target concentrations for the low to highest doe tested (HDT), respectively. Females were artificially inseminated.

Maternal signs such as muscle tremors, unsteadiness/ incoordination and increased respiratory rate were seen 4 hours after dosing and in some cases persisted for more than 24 hours at the HDT. No toxic signs were noted at the MDT and LDT. At the MDT one low and 3 control females were found dead or moribund from a mid-ear disease or respiratory infection. Test material related mortalities at the HDT occurred mostly prior to dosage reduction to 1.5 mg/kg. Nine of 22 animals survived to termination at the HDT. Two animals aborted at the MDT. No test material related body weight changes were noted.

No dose related soft tissue or skeletal anomalies were noted at any dose levels.

The NOEL/LOEL for dams were 1.0/1.5 based on tremors, unsteadiness/incoordination and increased respiration. The NOEL/LOEL for developmental toxicity were >3.0/>3.0 mg/kg/day.

The study is acceptable for Guideline 83-3 for a developmental toxicity study in rabbits and was upgraded from supplementary to fully acceptable in HED Doc# 004698 and by the RfD/QA Peer Review Committee.

Two-Generation Reproductive Toxicity Study/Rats (83-4)

CITATION: Astroff, A Barry (1997) A Two Generation Reproductive Toxicity study with Disulfoton Technical (Disyston ®) in the Sprague Dawley Rat. Laboratory name Bayer Corp., Stilwell, KA. Laboratory report number: 95-672-FZ, report# 108002, File 8368. November 19, 1997. MRID# 44440801. Unpublished

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRID# 44440801) disulfoton, technical, 99% a.i.] was administered to 30 Sprague Dawley rats/sex/dose in the diet at dose levels of 0, 0.5, 2.0 or 9.0 ppm (0, 0.025, 0.10 or 0.45 mg/kg/day by std. tables). Dosing was continuous for the P0 and F1 generation. Only one littering/animal/group was conducted. In this second 2-generation reproductive toxicity study with disulfoton, cholinesterase activity was measured in adults during pre-mating (at 8 weeks) and at termination and in pups at postnatal day 4 and day 21 in the 2 generations.

The major effects noted were cholinesterase inhibition and dams with no milk. In P0 males, plasma cholinesterase (PCHE) was significantly depressed and dose related pre-mating at 9.0 ppm (≥-34%) and at termination at 2.0 (≥-11%) and 9.0 ppm (-46%). In P0 females, plasma

cholinesterase (PCHE) was significantly depressed pre-mating (≥-29%) and at termination (≥-52%) at ≥2.0 ppm. In P0 males and females erythrocyte cholinesterase (ECHE) was significantly depressed and dose related at ≥2.0 ppm (≥-38% & ≥-35% males and ≥-46% & ≥-80% females) a pre-mating and termination, respectively, but only in females at termination (≥-14%) at ≥0.5 ppm. In P0 males and females brain cholinesterase (BCHE) was significantly depressed and dose related at ≥2.0 ppm in males (≥-11%) and ≥-14% in females at ≥0.5 ppm.. PCHE and ECHE depression in F1 males and females followed a similar nominal pattern to that in P0 males and females, except that the statistical significance varied within the F1 between two dose levels; sometimes the dose level showing statistical significance was higher and sometime lower of the two. In F1 males and females, BCHE was significantly depressed and dose related at ≥2.0 ppm in males (≥-14%) and in females (≥-50%). In F1 and F2 male and female pups at day 4 and/or day 21 of lactation, PCHE and ECHE were significantly depressed at 9.0 ppm. Values for PCHE and ECHE, respectively were at day 4 or day 21 in F1 male pups were (-24% & -47%) and for F1 female pups (-31% & -43%). Values for PCHE and ECHE, respectively, were at day 4 or day 21 in F2 male pups were (-46% & -53%) and for F2 female pups (-48% & -51%). In F1 and F2 male and female pups BCHE was significantly depressed at day 4 and day 21 at 9.0 ppm only (day 4 = -14% F1 males and -17% F1 females)(day 21 = -19% F1 males and -23% F1 females)(day 4 = -11% F2 males and -13% F2 females)(day 21 = -35% F2 males and -37% F2 females).

Muscle fasciculation (1 P0 female), tremors (15 P0 females, 10 F1 females) and dams (7 F1 dams) with no milk were noted at 9.0 ppm. No treatment related organ weight changes or histopathology were noted in P0 or F1 males or females at any dose level.

Clinical observations indicate that dams were not caring for their pups. Observed affects in pups in the 9.0 ppm group included 12 F1 (2 dams) pups cold to the touch and 3 F1 (2 dams) not being cared for and 63 F2 pups (7 dams) with no milk in their stomachs and 93 F2 weak pups (10 dams) from the affected dams. In addition, 1 P0 dam was salivating and gasping and did care for the litter and the litter died at 2.0 ppm. This effect at 2.0 ppm was considered test material related by the summary author of the 6(a)(2) submission (See summary 6(a)(2) report, MRID# 44440801; memorandum from David Anderson to PM 53, dated March 24, 1998, D242573), but ignored in the final report summary. Findings at necropsy were noted in F2 pups at 9.0 ppm that were expected in view of the maternal toxicity at this dose level. The report reasonably considered the pup deaths due to failure of maternal care, because of the weak and cold to the touch pups and failure of the pups to show milk in their stomachs. On careful examination of the report, this reviewer agrees with this conclusion. Thus, under these conditions, the effects in pups were caused by maternal toxicity and not the direct toxicity of disulfoton on pups.

Body weight change was lower than control values during gestation in P0 (-9%) and F1 (-15%) females. Body weights were significantly reduced at termination from control values in P0 (-6%) and F1 females (-13%) and in F1 males (-8%). No other significant body weights or changes were noted.

The P0 parental LOELs were 0.5 ppm (0.025 mg/kg/day) based on brain cholinesterase activity depression in P0 females with tremors and muscle fasciculation at 9 ppm in females during gestation and lactation from both generations and with body weight

decrements at 9.0 ppm, especially at termination. A NOEL of 0.5 ppm (0.025 mg/kg/day) was seen in F1 parents. F1 and F2 pup (4 day and 21 day old) cholinesterase activity, including brain cholinesterase activity was depressed only at 9.0 ppm (0.45 mg/kg/day) with 2.0 ppm (0.10 mg/kg/day) being the NOEL. The F1 pup NOEL/LOEL were 2.0/9.0 ppm (0.10/0.45 mg/kg/day) based on treatment related pup deaths and pup weight decrements at 9.0 ppm, probably from inadequate maternal care.

The reproductive study in the rat is classified acceptable and does satisfy the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4) in rat.

Two-Generation Reproductive Toxicity/Rats (83-4)

CITATION: Hixson, EJ and Hathaway, TR (1986) Effect of disulfoton (Di-Syston®) on reproduction in the rat. Conducting laboratory: Mobay Chem. Date: 2/12/86. Study# 82-671-02. MRID# 00157511. Unpublished Study.

EXECUTIVE SUMMARY: In an acceptable 2-generation reproductive toxicity study (MRID# 00157511; HED Doc# 011959 & 005796), disulfoton, technical (97.8%) was administered at 0, 1, 3 or 9.0 ppm (0, 0.04, 0.12 or 0.36 mg/kg/day). In this first and older reproduction study cholinesterase activity was measured in pups, but not in adults. In this first study of reproductive toxicity, the parental toxicity NOEL/LOEL were 3/9 ppm or 0.12/0.36 mg/kg/day based on nominally reduced incidence of females with sperm and reduced body weight in gestating and lactating P0 females with cholinesterase being probably inhibited with a NOEL/LOEL of 1/3 ppm or 0.04/0.12 mg/kg/day. These latter cholinesterase results were supported by results from the chronic/oncogenicity rat study. Toxicity on reproduction showed a NOEL/LOEL of 1/3 ppm or 0.04/0.12 mg.kg/day based on F1a weanling pup brain cholinesterase inhibition and F2b pup survival.

The study is acceptable for a guideline (83-4) study on reproduction in the rat.

GENE TOXICITY TESTING: The following was taken from a document written by Nancy McCarroll for the Hazard Identification Assessment Review Committee proceedings. Combining the acceptable studies with the additional EPA-sponored studies will satify the Pre-1991 mutagenicity initial testing battery guidelines. No further mutagenicity testing has been identified at this time. In addition, disulfoton is not genotoxic *in vivo* or carcinogenic in mice or rats.

In some of the mutagenicity studies, positive effects were seen without activation while negative effects were seen with activation. This may be due to microsomal enzyme metabolism, since pretreatment of rats and mice with phenbarbital reduces toxicity from disulfoton.

Gene Mutation (84-2)

MUTAGENICITY: *Salmonella typhimurium/Escheerichia coli* reverse gene mutation plate incorporation assay (Accession No. 00028625; Doc. No. 003958: As part of an Agency sponsored mutagenicity screening battery, disulfoton was negative in all strains up to the HTD (5000 μg/plate +/- S9) in three independent trials.

MUTAGENICIY: Chinese hamster ovary (CHO) cell HGPRT forward gene mutation assay (MRID# 40638401, Doc# 008394): This unacceptable study is considered to be positive, because the assay was conducted at partially soluble levels(0.1-1.0 μL/ml -S9; 0.7-1.0 μL/ml +S9) and insoluble doses (5-10 μL/ml -S9; 3-10 μL/ml +S9) but not active at soluble concentrations ($\leq$0.06 μL/ml +/-S9). The mutagenic response appeared to be stronger without metabolic (S9) activation .

Chromosome Aberrations (84-2)

CITATION: Micronucleus Test on the Mouse, performed by Bayer AG, Wuppertal (Germany), Study No. T2059008/Bayer Final Report No. 23887, dated January 13, 1995. (MRID No. 43615701). Unpublished. report.
Conclusions: This study is judged acceptable, as demonstrating no increase over background in micronucleated polychromatic erythrocytes (evidence of cytogenetic damage) of mice treated intraparitoneally up to MTD levels (8 mg/kg). Lethality and other signs of toxicity, but no bone marrow cytotoxicity was seen.

Other Gene Mutations: (84-2)

Bacterial DNA Damage/Repair: *E Coli* DNA damage/repair test (Accession# 072293; Doc# 004698): The test is negative up to the HDT (10,000 μg/plate +/- S9.

Mitotic Recombination: *Saccharomyces cerevisiae* D3 mitotic recombination assay (Accession# 00028625; Doc# 003958): Disulfoton (up to 5% +/- S9) was negative at this endpoint in the Agency-sponsored mutagenicity screening battery. The study is currently listed as unacceptable, but should be upgraded to acceptable. Upon further review of the data, it was decided that the reason for rejecting the study (number of replicates/dose not provided) did not interfere with the interpretation of the findings.

Sister Chromatide Exchange: Sister chromatide exchange in CHO cells (MRID# 4095001; Doc# 008394): Positive, dose related effects at 0.013-0.1 μL/ml without S9, but not active in the S9 activated phase of testing up to a level (0.20 μL/ml) causing cell cycle delay.

Sister Chromatide Exchange: Sister chromatide exchange in chiniese hamster V79 cells (Accession# 072293; Doc# 0044223): The test is negative without activation up to the HTD (80 μg/ml).

Subsequently tested by the same investigators (Chen et al., 1982; Environ. Mutagen. 4: 621-624) in the presence of exogenous metabolic activation and found to be negative up to the HDT (80 μg/ml).

Unscheduled DNA Synthesis (UDS): UDS in WI-38 human fibroblasts (Accession# 000028625; Doc# 003958): The test is positive in the absence of S9 activation at percipitating doses (1000-4000 μg/ml). With S9 activation, the study was negative at comparable percipitating concentrations.

Other EPA Sponsored Studies:

Disulfoton was also included in second tier mutagenicity test battery performed at the EPA (EPA-600/1-84-003) in 1984. Although DERs have not been prepared for these additional assays, we assess that they are acceptable for regulatory purposes.

Mouse Lymphoma L5178Y TK+/- forward gene mutation assay: The test was positive in the absence of S9 activation with concentration dependent and reproducible increases in mutation frequency at 40-90 μg/ml; higher dose levels were cytotoxic. No mutagenic activity was seen in the presence of S9 activation up to a cytotoxic dose (150 μg/ml).

Mouse Micronucleus Assay: The test is negative in Swiss Webster mice up to a lethal dose (8 mg/kg) administered once daily for 2 consecutive days by intraperitoneal injection. No bone marrow cytotoxicity was seen.

Sister Chromatide Exchange in CHO cell assay: The nonactivated test was negaitve up to levels ($\geq$0.02%) that caused cell cycle delay, but the test material was weakly positive at a single dose (0.04%) with metabolic activation.

Metabolism: (85-1)

CITATION: Lee, SGK, Hanna, LA, Johnston, K and Ose, K (1985) Excretion and Metabolism of Di-syston® in Rats. Study# 90946. Dated December 9, 1985, September 20, 1988, May 17, 1990 seotember 26, 1990 and April 29, 1992. Conducted by Mobay Corp. MRID# 42565101.

EXECUTIVE SUMMARY: The aborption, distribution, metabolism and excretion of Di-systion® were studied in groups of male and female Sprague Dawley rats administered a single dose of 0.2 or 1.0 mg/kg Di-syston® - ethylene-1-^{14}C, or a 14-day repeat oral dose of 0.2 mg/kg unlabeled Di-Syston® followed by 0.2 mg/kg [^{14}C]-labeled Di-Syston® on day 15. [^{14}C]-Di-Syston® was rapidly absorbed, distributed, metabolized completely and eliminated in rats under all dosing regimens. Over 95% of the recovered label was excreted in the urine in all groups, and excretion was approximately 90% complete within24 hours of dosing. Less than 2% of the recovered label was in the feces. Bioaccumulation was also not observed, with $\leq$0.3% of the radiolabel recovered in

the tissues and ≤1% in the carcass.

A major metabolite (43-60% of the radioactivity in the urine) and a minor metabolite (6-20% of the urinary radioactivity) were produced by hydrolysis of oxidative metabolites. These metabolites were identified as sulfonyl [1-(ethylsulfonyl)-2-(methylsulfinyl)ethane] and sulfinyl [1-(ethylsulfinyl)-2-(methylsulfinyl)ethane], respectively. Three minor oxidative metabolites (Di-Syston sulfone, Di-Syston oxygen analogue sulfoxide, and Di-Syston oxygen analog sulfone) were identified. Sex-related differences in pattern of these metabolites and differences between the single dose and the repeat dose groups were attributed to differences in metabolic rates, rather than different metabolic pathways. A metabolic pathway for Di-Syston was proposed.

Study classification: The study is classified as acceptable. The study satisfies the registration requirements under Guideline 85-1 (and Addendum 7) for metabolism in rats. Althjough there were minor deficiencies in the study, they did not affect the overall study results and conclusion (see Reviewer's Discussion, Section E). A metabolite was not fully characterized, however, the testing laboratory inducated that after using different solvents the metabolite co-chromatographed with a oxygenated hydrolytic product of disulfoton, 1-(ethylsulfonyl)-2-(methylsulfinyl)ethane and material at the origin co-chromatographed with 1-(ethylsulfinyl)-2-(methylsulfinyl)ethane.

Dermal Absorption/Rats (85-3)

CITATION: Warren, D.L. (1994) Dermal Absorption of ^{14}C-Disulfoton from the DISYSTON 8 Formulation. Miles, Stilwell, KS. Study No. 94-722-YP. August 30, 1994. MRID 43360201. Unpublished.

EXECUTIVE SUMMARY: In a dermal absorption study (MRID 43360201) ^{14}C-Disulfoton (99.3% a.i., Specific activity 53 mCi/mmol; cold disulfoton 86.5% a.i.) in 150μl emulsion was applied to clipped backs (≈ 15 cm^2 area) of 4 male rats/dose/group at dose levels of 0.85, 8.5, and 85 μg/cm^2 for 1, 4, and 10 hours (MRID# 43360201). At the 10th hour all the skins were washed to terminate the exposure. At the termination of exposure, these animals were kept for an additional 158 hours to determine kinetics of absorption and excretion of the material remaining on/in the skin following washing. Following the application of the material, the rats were placed individually in metabolism cages and total urine and feces collected separately. Following the wash of the application site, the urine and feces were collected in 24 hour aliquots.

Disulfoton is well absorbed and about 31 - 37% and 2.7 - 3.3% of the administered dose was excreted in the urine and feces, respectively. Ten to 30% of the applied dose evaporated during the 10 hours exposure period in all groups. Skin residues as percent of administered dose increased with dose and decreased with time in all groups. The % absorbed increased with time, essentially equal with time. At low dose, the % absorption at 1, 4, and 10 hours was 5.9, 13.7 and 26%; at mid dose it was 4.6, 15.9, and 32.7%; and at high dose 3.6, 12.5 and 25.6%, respectively.

The study is classified as **Acceptable** and satisfies the guideline requirement for dermal penetration study (85-3) in the rat.

Special 6-Month Cholinesterase Study (No Guideline#)

CITATION: W.R. Christenson, B.S. Wahle (1993) Technical grade disulfoton (Di-Syston®): A special 6-month feeding study to determine a cholinesterase no-observed-effect level in the rat. Study# 91-972-IR, (12/3/1993), conducted at Miles Inc., Agricultural Division, Toxicology Stilwell, Kansas for Miles Inc., Agricultural Division, Kansas City, Missouri. MRID No.: 43058401. Unpublished Report.

EXECUTIVE SUMMARY: In a 6-month study designed to establish a NOEL and LOEL for cholinesterase inhibition, technical grade disulfoton (98-99% pure) was administered in the diet to 35 male and female Fischer 344 rats for up to 6 months at levels of 0, 0.25, 0.5 or 1 ppm (approximate doses of 0, 0.02, 0.03 or 0.06 mg/kg/day for males and 0, 0.02, 0.03 or 0.07 mg/kg/day for females)(MRID# 43058401). At the end of 2, 4 and 6 months, 10 rats/sex/dose were taken for blood and brain cholinesterase assays.

Statistically significant inhibition of cholinesterase activity was observed in erythrocytes in females at all doses (3-14% inhibition, 11-17% inhibition, and 23-29% inhibition at 0.24, 0.5, and 1.0 ppm, respectively. In addition, at 1.0 ppm, males had decreased erythrocyte cholinesterase activity (10-16% inhibition) and females had decreased plasma (8-17% inhibition) and brain (7-13% inhibition) cholinesterase activities. However, biologically significant and statistically significant inhibition of cholinesterase activity was observed only in the plasma, erythrocytes and brain of females at 1.0 ppm. No biologically significant inhibition of cholinesterase activity was observed in males.

The LOEL for inhibition of cholinesterase activity was 1.0 ppm is based on a 23-29% inhibition of erythrocyte, 12-17% inhibition of plasma and 13% inhibition of brain cholinesterase in females. The NOEL is 0.5 ppm (0.03 mg/kg/day). No biological meaningful cholinesterase inhibition was observed in males at any dose level.

Body weight, food consumption, and clinical signs were also monitored, but showed no treatment related effects. **Based on these few parameters, no systemic effects were observed at any dose level and the NOEL for systemic toxicity was 1.0 ppm (0.06 mg/kg/day for males and 0.07 mg/kg/day for females).**

Core classification: The special non-guideline study is acceptable for the requested 6-months cholinesterase study in rats.